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# Some Clinical Uses of Hormones in the Cow

by

Mylon E. Filkins\*

## INTRODUCTION

The clinical use of hormones for the augmentation of fertility in the female is of practical importance. To the Livestock breeder, the value of his stock is measured by the production of offspring. It is essential that the veterinarian have an understanding of the complex hormonal control of the estrus cycle. This knowledge is necessary to understand possible variations in length and activities of the cycle and aids in the therapeutic use of hormone products.

This paper is not intended to discuss the complex hormonal mechanism of the female, but rather is designed to discuss the clinical application of hormones in the augmentation of fertility.

For sake of brevity, the following terms will be used: pregnant mare serum (PMS); human chorionic gonadotropin (HCG); follicle stimulating hormone (FSH); luteinizing hormone (LH). It should be kept in mind that although PMS contains mostly FSH, it also contains small amounts of LH. HCG consists largely of LH, but does

contain small amounts of FSH. Pituitary gonadotropin contains mostly FSH and some LH.

FSH and LH are normally secreted by the anterior pituitary gland. FSH stimulates the growth of ovarian follicles in the mature female and stimulates sexual development in the immature females. LH stimulates the interstitial tissue of the ovary and promotes the growth of the corpus luteum. The corpus luteum responds to LH stimulation by the production of progesterone. Progesterone depresses the myometrium sensitivity to estrogens and oxytocin, and prepares the uterine mucosa for fetal attachment. Progesterone suppresses FSH and LH production by the anterior pituitary and has a general counter-estrogen effect. Estrogens are produced by the ovarian follicle and the placenta of pregnancy. Since FSH stimulates follicle growth, it indirectly stimulates the liberation of estrogens. The natural estrogens, estrone and estriol, stimulate and maintain female primary and accessory reproductive organs. Estrogens also promote uterine muscle tone and sensitivity of the uterus to oxytocin, as well as increasing the vascularity and se-

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cretions of reproductive organs. Estrogens promote mating behavior or the clinical signs of estrus.

Herrick (1963) has listed the following words of caution on hormone therapy.<sup>11</sup> These should be kept in mind when ever hormone therapy is considered.

DON'T:

1. Use hormones until pregnancy is confirmed.
2. Use progesterone for ovulation failure.
3. Use estrogens alone for infantilism.
4. Use FSH where LH is indicated.
5. Overdose.
6. Use estrogens or progesterone where gonadotropins are indicated.
7. Always expect results from one treatment.
8. Use hormones without a thorough examination of the reproductive tract.
9. Treat animals of such relatively minor value that owners become reluctant to permit treatment of those of economical importance.
10. Use hormone therapy until after presenting a proper diagnosis and emphasizing management recommendations.

### **CYSTIC OVARIES**

#### ***Etiology and Pathogenesis***

Cystic ovaries is principally a disease of dairy cows; however, beef cows may be similarly affected. Cystic ovaries are thought to be caused by an abnormal endocrine function of anterior pituitary origin. The FSH-LH balance is upset with an oversecretion of FSH and an undersecretion of LH. LH may never reach a level high enough to bring about ovulation. This inhibition of ovulation may cause the persistent maintenance of a large follicle in the ovary or continuing degeneration of several of the follicles which do not ovulate. If there is enough LH present to cause ovulation, there may not be enough present to cause normal luteinization of the follicle. The excessive secretion of estrogens by the cystic ovarian follicle elicits the clinical symptoms of nymphomania. There have been many theories advanced as to the cause of the pituitary imbalance. Excessive prolactin produc-

tion, adrenal malfunction, thyroid malfunction, inhibition of the release of LH by the hypothalamus, and length of day have all been proposed as causing the endocrine imbalance.

#### **CLINICAL SIGNS**

Cystic ovaries affect cows of all ages, but are most commonly seen during the second to fifth parturition. The time of onset of cystic ovaries is usually from one to four months after calving. This is the time that milk production is the highest; thus the theory of prolactin causing the endocrine imbalance has been advanced. There is no direct evidence that cystic ovaries are an inheritable condition, but many investigators propose a hereditary predisposition to the disorder.

Cystic ovaries may cause three types of animal reaction: continued estrus behavior (nymphomania); no estrus (anestrus); and cyclic estrous periods which appear to approach normality. The behavior of the nymphomaniac is most dramatic and the most common (75%) in cows with cystic ovaries. Due to the influence of high levels of estrogens, the vulva becomes hyperemic, the relaxation of ligaments permits the pelvis to tilt forward resulting in a high tail head. The cows are often nervous, restless and bellow frequently. The cow may occasionally accept the riding of another cow, or coitus by the bull at any time. Nymphomaniac cows are often as sexually aggressive as a bull in seeking out and attempting to mount a cow approaching or in estrus. In chronic untreated conditions, the nymphomaniac cow will develop a characteristic steer-like appearance and may progress to an anestrus state.

The occurrence of cystic follicles with the complete absence of estrus is less frequent and much more difficult to detect from external appearance of the cow. Usually these cows have a history of lack of estrus for 1 to 4 months or more. This period of anestrus is frequently confused with pregnancy by the owner.

The third type of reaction to cystic ovaries, occurs in cows with periodic estrus behavior, which at times seems normal. Some of these cows may act as if they

were in estrus, but refuse to stand for the bull or other cows. Apparently the cysts go through cycles of development, one will grow, develop to a point and then regress. As the first one regresses a second one will develop and estrus will occur as the cyst reaches a certain stage of development.

#### **DIAGNOSIS**

Diagnosis of cystic ovaries is based on the breeding history and clinical signs of the animal. A rectal palpation should be performed to determine the state of the ovaries and whether or not cystic follicles are present. One to four cysts varying in size from .75 to 3 inches in diameter may be palpated on one or both ovaries. The cysts are usually peripheral in location, thin-walled and burst when pressure is applied. It may be difficult to differentiate between a small cyst and a normal follicle. Repeated examination, a vaginal examination, and history will be of value in differentiating cystic ovaries from other conditions. The uterus should be checked for pregnancy in all cases to avoid an error in diagnosis or therapy.

#### **TREATMENT**

There is some disagreement as to the preferred treatment of cystic ovaries. Roberts (1956) has found progesterone to be of limited value while Beck and Ellis (1960) advocate its use. Even though FSH would seem to be contra-indicated, Nishikawa and Sugie (1962) propose the use of PMS and HCG in treating cystic ovaries. Ellis and Beck (1960) investigated the use of repositol progesterone (Pitman Moore) at a dose of 1 mg./lb. body wt.<sup>2</sup> In 100 cows studied with ovarian cysts and clinical signs of nymphomania excessive estrus ceased 36–72 hours after the intramuscular injection of progesterone. Palpation revealed normal gradual ovarian changes, either with the formation of the corpus luteum of pregnancy, or return to the pro-estrous stage with development of Graaphian follicles and then estrus. Pregnancy resulted in 67 of the cows at the first breeding and 33 of the cows conceived after more than one service. As mentioned earlier, normally the uterus is prepared for the implantation and nour-

ishment of the embryo under the influence of progesterone secreted by the corpus luteum. This influence is lacking in the cow with cystic ovaries and conception is prevented.

Nishikawa and Sugie (1962) proposed the use of PMS and HCG in the treatment of nymphomaniac cows.<sup>13</sup> The purpose of the treatment was to develop many follicles temporarily in healthy tissues of a cystic ovary; to produce "artificial ovarian cysts"; and to make use of the process of rapid degeneration and absorption of these artificial cystic follicles. The dosage used was 8,850–13,550 m.u. (mouse units) of PMS; 6,000–13,500 I.U. PMS concentrate, and 5,000–6,000 I.U. PMS concentrate together with 2,500–3,000 m.u. of HCG. Many follicles developed temporarily (with all three treatments) in the healthy tissues of the cystic ovaries. Follicles began to develop 2–5 days after injection. They gradually or rapidly increased in number and increased the ovarian volume so that the entire ovary resembled a mass of follicles. The new follicles were absorbed along with the cysts and the ovary recovered its normal function within 20 to 40 days after the PMS injection. In many cows (11 of 13), normal estrus occurred. Estrus was repeated in normal cycles and 16 of 20 inseminated cows conceived. The use of PMS has several disadvantages in that a fairly long period of 20–40 days elapsed from injection to recovery of normal ovarian function; lactating cows responded to PMS injections with a drop in milk production for 7–10 days and the milk was coagulated in some cases.

The "old" treatment for ovarian cysts consisted of repeated manual rupture or removal of the cysts at 6–10 day intervals until a normal cycle and corpus luteum developed. This technique usually traumatizes the ovary and should be avoided.

Tapping of the cyst or nicking with a scalpel through the vaginal wall has been employed. Spaying will correct nymphomania; however the remaining ovary will often become cystic.

Roberts (1956) recommends the use of HCG intravenously or intramuscularly.<sup>14</sup> In 127 cases of cystic ovaries treated with 2500–5000 I.U. HCG intravenously, 83.8%

recovered and 75.6% conceived. In 37 cases treated with 10,000 I.U. HCG intramuscularly, 81.6% conceived. Roberts found only 13 of 27 cases responded to PMS injections 1000–2000 R.U. (rat unit) S.Q. This is in contradiction to the work of Nishikawa and Sugie (1962) who reported 80% conception.<sup>13</sup> The higher dose of PMS used by the latter investigators probably reconciles the disagreement.

### **INFANTILISM or HYPOPLASIA OF THE REPRODUCTIVE TRACT**

Infantilism or hypoplasia of the reproductive tract may be seen as the result of intensive inbreeding. The disorder is due to a deficiency in pituitary gonadotropins during development. The condition is genetically linked; however, there is no breed predisposition.

Diagnosis can be made by rectal palpation in that the ovaries may be very small or even undetectable. The female reproductive organs are in an adolescent state with little or no mammary development. The heifers do not come into estrus and do not ovulate functional ova if ovulation does occur.

Treatment is usually useless; however, in unusual cases full physiological doses of 50 mg. FSH or 1,000–2,000 I.U. PMS are indicated.<sup>11</sup> Estrogens are contraindicated. This condition may require several weeks or months to correct and repeated injections are required.

### **ESTRUS SYNCHRONIZATION**

With the successful use of artificial insemination, the practical value of estrus synchronization has increased. The grouping of the calf crop can provide several advantages such as: a more uniform calf crop, optimum use of labor, equipment and management, and better utilization of foodstuffs.

Nearly all reproductive hormones have been employed in regulation of female reproduction. However, many are impractical or are not effective. Compounds for control of estrus must meet several requirements before they can be recommended for use. They must: (1) control estrus and ovulation when administered at dif-

ferent reproductive stages; (2) be effective at dosage levels which produce predictable results; (3) effectively synchronize estrus and ovulation; (4) not impair fertility; (5) permit a uterine environment compatible with embryonic survival and (6) not interfere with later reproductive potential.

To date the orally effective synthetic steroids with progestational activity show the most practical results. Such a compound, 6-chloro- $\delta$ -6-17-acetoxypregesterone (CAP) has been found to be orally effective for inhibiting estrus and ovulation in dairy heifers and cows at relatively low daily doses.<sup>4</sup>

Another similar compound, 6-methyl-17-acetoxypregesterone (MAP) has also been proven effective for the inhibition of ovulation in dairy and beef heifers.<sup>1</sup>

The oral hormones are usually contained in a premix (i.e. soybean meal), which is mixed with ground ear corn or other feeds. It is important that the feed be well mixed and that all animals receive a share of the feed. The experimental results of orally administered MAP, CAP, and CAP plus 17-estradiol-3-methyl ether (3-ME) on estrus and conception have been studied.<sup>1, 4</sup> Estrus was effectively suppressed in all treated animals during a 20 day feeding period. The heifers receiving MAP at levels of 150 and 210 mg. per day returned to estrus within three to six days following hormone withdrawal. Conception rate at the controlled estrus was similar to that observed in a control group (55% vs 60%). Heifers receiving 5 mg. CAP exhibited estrus four to eight days after withdrawal of the hormone, while eight of ten animals treated with 5 mg. CAP and 10 mg. 3-ME were in estrus four to six days after the end of treatment. Estrus was observed in 11 of 15 heifers treated with 10 mg. CAP four to eight days following hormone withdrawal. Conception rates on the first post-treatment estrus were lower than those observed in control groups. A conception rate of 42 to 50% at the first post-treatment estrus has been reported. The addition of estrogen to the 5 mg. of CAP had little effect on estrus response or fertility. Normal estrous cycle intervals occur after the adjusted

estrus and fertility is equal to that of controls.

A mixture of norethynodrel and 1.5% 3-methyl ether of ethynylestradiol (NE) has inhibited estrus and ovulation during the treatment period by daily subcutaneous injections or oral administration. The injected heifers returned to estrus three to seven days after post-treatment with 44% conceiving at the adjusted heat. Orally treated heifers returned to estrus 3–24 days post-treatment and 75% of the heifers conceived at the first service. A single injection of NE in a repositol preparation was less effective in synchronizing cycles and conception rates were reduced.

### REPEAT BREEDING

A cow may have a normal reproductive tract, regular estral cycles, no evidence of disease, and yet fail to settle. Frequently, these animals have a recurrence of estrus in 25–45 days after service. The cause for the poor conception rate may be due to a small corpus luteum and a resultant deficiency in progesterone. Without adequate progesterone, pregnancy cannot be maintained and fetal reabsorption may occur.

The use of progesterone in repeat breeding of heifers can increase conception rates. Herrick (1953) studied forty heifers with a history of repeated breeding and a normal clinical examination.<sup>10</sup> Twenty of the heifers were injected with 500 mg. of repositol progesterone at the time of service; 13 of them became pregnant (65%). Only four of 20 controls became pregnant (20%). Dawson (1954) has also proposed the use of progesterone in repeat breeding.<sup>3</sup> He found that 36 of 47 heifers with long histories of infertility conceived after treatment with 100 mg. progesterone by implantation. 18 controls in the same herd failed to conceive after five matings.

### ANESTRUS

(No Significant Structure on Either Ovary)

Inactive ovaries are found in cows under various forms of stress. The condition commonly occurs in high-producing dairy cows; in cows nursing more than

one calf; and as an aftermath of metritis, overcondition, malnutrition and phosphorus deficient diets. It is probable that the cows known as "easy keepers" may actually have a thyroid deficiency. These cows are often barren and this may be due to inactive ovaries resulting from the hypothyroidism.

This condition should be differentiated from infantilism or hypoplastic ovaries. In the latter, there is no history of pregnancy while in anestrus, the animal usually has a history of pregnancy. Rectal palpation will reveal a normal reproductive tract and no ovarian activity in the anestrus animal.

The hormonal therapy for this type of anestrus may include the thyroid hormones. Herrick (1963) recommends 5 gr. of thyroid extract daily for a week; he also recommends that iodinated casein be fed.<sup>11</sup> Implants of triiodothyronine have been used successfully in the barren mare. It is possible that 10–15 mg. triiodothyronine implants may be effective in the cow.

Estrogens are also used in the treatment of anestrus; however, it is wise to determine via rectal palpation the condition of the ovaries before estrogens are administered. Ovarian cysts would contraindicate the use of estrogens. Gibbons (1964) recommends 25–30 mg. injections of diethylstilbestrol for anestrus cows.<sup>6</sup> He found that a single injection of stilbestrol brought 80% of the cows into estrus with an 80% conception rate if bred at the induced estrus. A second injection of stilbestrol was found to bring an additional 15% into estrus. Herrick, (1963) reported the following dosages of estrogens: stilbestrol 25–30 mg.; estradiol-17 cyclopentyl propionate (ECP) (Upjohn) 4–5 mg.; and V-Estrovian (Warren-Teed) 1–2 cc.<sup>11</sup>

Gibbons (1964) also reported on the use of FSH in treating anestrus cows.<sup>6</sup> A dose of 15 mg. FSH induced estrus in 29 of 48 anestrus cows. Of these 29 cows, 12 conceived when bred at the induced estrus. A dose of 50 mg. FSH used on 12 anestrus cows induced estrus in five of which two became pregnant after being bred at the induced estrus.

## ANESTRUS

### (Luteal cysts) and "Silent Estrus"

Luteal cysts are common, particularly in cattle on grass low in estrogens and/or accompanying metritis. The condition may not be easily distinguished by rectal palpation as there may be a normal corpus luteum present. The animal with a retained corpus luteum is difficult to differentiate from the animal with "silent" estrus. In both cases, there may be anestrus for 90–120 days following calving. Rectal palpation at weekly intervals can be used to detect "silent" heat. It is also possible that by using better methods, the estral cycle of the "silent" cow can be detected.

Herrick (1963) recommends that care be exercised in deciding to express the "retained corpus luteum."<sup>11</sup> He recommends manual enucleation followed by 20 mg. of stilbestrol to sensitize the tract, followed in 12 hours by 50 mg. FSH. Roberts (1956) claims that animals with luteal cysts will recover without treatment and cites substantial evidence for his statement.<sup>14</sup> In 94 cows, where the corpus luteum was removed, 69% conceived; in 78 cows given 1000 I.U. PMS, 76% conceived, in 64 control animals, 78% conceived. However, most veterinarians and farmers feel that something should be done so the corpus luteum is expressed or the animal is given hormones.

Herrick (1963) suggested the use of 20–25 mg. FSH 2–3 days prior to anticipated estrus in cows with a "quiet" estrus.<sup>11</sup> The FSH stimulates follicular growth; follicular fluid liberates estrin, which promotes estrus activity. In the cow in which "silent" heat has been detected, the injection of 10–15 mg. of stilbestrol at the onset of estrus will induce behavioral estrus.

### INDUCTION OF SUPER-OVULATION IN CATTLE

The successful transfer of fertilized ova from one female to another has been accomplished in several species. The application of this technique may have practical value in the propagation of desired blood lines or in production testing. It is possible that by inducing multiple ovula-

tions in the cow and transferring fertilized ova to "foster cows" that animal breeding and selection could progress at a much faster rate.

The induction of super-ovulation in the bovine is based on the assumption that a ripe follicle begins to develop during or just after the last estrus. There is reason to believe that this follicle grows with others which become atretic. If this is so, super-ovulation can be induced by a process which prevents atresia or stimulates the growth of new follicles.

Schilling and Holm (1963) have investigated the induction of limited multiple ovulations in cattle.<sup>16</sup> On the fifth day after heat, a single dose of 1000–1500 I.U. PMS was given to eleven cows. None of the animals exhibited estrus following this injection. The corpora lutea were enucleated between the 16th and 18th day, followed by a dose of 2000 I.U. PMS. After 3–5 days all animals came into heat and were given 4000 I.U. LH intravenously. The cows were slaughtered 48–96 hours after ovulation. Four of the cows had ovulated 2 ova; four cows had ovulated 3 ova; 1 cow had released a single ovum and two cows failed to ovulate. There was an average of only three small follicles on the ovaries. There were no histological defects noted on fifteen eggs examined and fourteen of the fifteen eggs had been fertilized. In the three cows which did not receive PMS on the fifth day, there was an average of 16 small follicles on the ovaries with only 1–3 ova being ovulated.

Hafez and co-workers (1963) induced superovulation in eighty Hereford heifers using PMS and HCG.<sup>7</sup> The heifers were given 3000–5000 I.U. of PMS in one, two, or three doses, administered on day 12, 14, 16, and 18 of the estrous cycle, with or without enucleation of the corpus luteum. 2000 I.U. HCG were given 3, 4, 5, 6, or 7 days after the first PMS injection. The animals were bred and slaughtered 2 to 5 days after ovulation. It was found that the mean number of mature follicles and percentage that ovulated were almost identical for the right and left ovaries. The development of palpable follicles

started 1–2 days after the first PMS injection; ovulation occurred when the follicles reached an average diameter of 14 mm. The number of ovulated follicles was not related to an increase in the volume of the ovary.

Superovulatory response was found to be greater in the ovaries where the corpus luteum was enucleated; however, no advantage was obtained by injecting the PMS earlier than the 16th day after estrus. The total number of follicles developed was higher at 5000 I.U. PMS than at 3000 I.U., but there was a substantial increase in the percentage of luteinized and hemorrhagic follicles at the higher dose.

As many as fifty-two ova were released per cow with an average of 20 ova being shed per cow; however, the fimbriae appear capable of picking up ova released from ten follicles (on an average). Of all the ova examined, 15% were considered to be morphologically abnormal.

Hafez and co-workers have investigated the effect of estrogens on superovulation.<sup>8</sup> 5, 10 or 20 mg. of 17- $\beta$ -estradiol was injected intramuscularly before, simultaneously with or following the injection of PMS. Estrogen injections were repeated for 2, 5 or 10 days. It was found that when estradiol was administered before PMS, there was a significant reduction in the number of developed follicles. However, if the estrogen was administered after the PMS, there was a significant increase in the number of developing follicles. The proportion of follicles which ovulated was not affected by estradiol. In heifers treated with estradiol before PMS, the number of fertilized ova per cow ranged from 6–17 with an average of 11.3 ova being fertilized.

### SUMMARY

Some of the hormonal treatments for infertility in the cow were discussed. True anestrus is best treated with 25–30 mg. stilbestrol. Infantilism is usually unresponsive to treatment; however, prolonged FSH therapy may be of benefit. Luteal

cysts may be manually expressed and treated with estrogens; however, treatment may not be necessary. Cystic ovaries are best treated with 5,000–10,000 I.U. of LH. Repeat breeding is combatted with 250 mg. progesterone 48 hours after estrus, repeating every 2 weeks until pregnancy is well established. "Silent" heat may be strengthened with the injection of 10–15 mg. of stilbestrol. MAP (150 mg./day) and CAP (5 mg./day) have been found effective in the synchronization of estral cycles. Superovulation may be induced by PMS injections on the 16th day of the cycle followed in 5–7 days by HCG injections.

It should be remembered that no hormone or combination of hormones known at the present time can be injected and depended on to produce *normal* maturation of the follicle, estrus, and ovulation with a *normal* fertile ovum.

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